1.0 Title

Standard Operating Procedure for Determination of Sulfide (Colorimetric, Methylene Blue) (6216)

2.0 Scope and Application

The purpose of this method is to determine the sulfide concentration. This method is applicable to the measurement of total and dissolved sulfides in drinking, surface and saline water and domestic and industrial wastes. This method is not applicable to acid insoluble sulfides such as copper sulfide. This method is suitable for the measurement of sulfide in concentrations from 0.1 to 20 mg/l.

3.0 Summary of Method

Sulfide reacts with dimethyl-p-phenylenediamine (p- aminodimethyl aniline) in the presence of ferric chloride to produce methylene blue, a dye which is measured at a wavelength maximum of 625 nm.

4.0 Definitions: The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

4.1 Dissolved Analyte: The concentration of analyte that will pass through a 0.45 um membrane filter assembly prior to sample acidification

4.2 Method Detection Limit (MDL): The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

4.3 Linear Dynamic Range (LDR): The concentration range over which the analytical curve remains linear.

4.4 Laboratory Reagent Blank (LRB): An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, reagents, and acids that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus

4.5 Stock Standard Solution: A concentrated solution containing one analyte purchased from a reputable commercial source. Stock standard solutions are used to prepare calibration solutions and other needed analyte solutions.

4.6 Water sample: For the purpose of this method, a sample taken from one of the following sources: surface, ground, storm runoff, industrial or domestic wastewater.

4.7 Units of weights and measures: g gram, mg milligram, ug microgram, l liter, ml milliliter, ul microliter.

4.8 May: This action, activity, or procedural step is neither required nor prohibited.

4.9 May not: This action, activity, or procedural step is prohibited.

4.10 Must: This action, activity, or procedural step is required.

4.11 Shall: This action, activity, or procedural step is required.

4.12 Should: This action, activity, or procedural step is suggested, but not required.

5.0 Interferences

5.1 Reduced sulfur compounds, such as sulfite, thiosulfate and hydrosulfite, which decompose in acid may yield erratic results.

5.2 Volatile iodine-consuming substances will give high results.

6.0 Safety

6. 1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable through using hoods, gloves and other appropriate personal safety equipment.

6.2 Precautions should also be taken to minimize other potential hazards. Basic good housekeeping and safety practices such as the use of rubber or plastic gloves, lab coat, and safety glasses during handling of samples and cleaning of labware are recommended.

6.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS)

should be available to all personnel involved with this method.

6.4 As with all electrical and heated instruments, observe basic safety rules. Do not touch electrical areas and allow surfaces to cool before touching.

7.0 Equipment and Supplies: Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.1 Matched test tubes, approximately 125 mm long and 15 mm O.D.

7.2 100 ul eppendorf. To obtain uniform drops, hold the eppendorf in a vertical position and allow the drops to form slowly.

7.3 Photometer: Spectrophometer for use at 625 nm with cells of 1 cm and 10 cm light path.

8.0 Reagents and Standards

8.1 Amino-sulfuric acid stock solution: Dissolve 13.5 g of N,N- dimethyl-p--phenylenediamine oxalate (p-aminodimethylaniline) in a cold mixture of 25 ml concentrated H_2SO_4 and 10 ml distilled water in a 50 ml volumetric flask. Cool and dilute to volume. If the mixture turns dark, discard and purchase fresh reagent. Store in a dark glass bottle.

8.2 Amino-sulfuric acid reagent: Dissolve 12.5 ml of amino-sulfuric acid stock solution in 487.5 ml of 1+1 H_2SO_4. Store in a dark glass bottle. This solution should be clear.

8.3 Ferric chloride solution: Dissolve 100 g of $FeCl_3 \cdot 6(H_2O)$ in 40 ml distilled water.

8.4 Sulfuric acid solution (H_2SO_4), 1+1.

8.5 Diammonium hydrogen phosphate solution: Dissolve 200 g of $(NH_4)_2HPO_4$ in 400 ml distilled water.

8.6 Methylene blue solution I: Dissolve 0.25 g of methylene blue in distilled water in a 250 ml volumetric flask and dilute to volume. Use U.S.P. grade or one certified by the Biological Stain Commission. The dye content reported on the label

should be 84% or more. Standardize against sulfide solutions of known strength and adjust concentration so that 50ul equals 1.0 mg/l sulfide.

8.7 Methylene blue solution II: Dilute 10.00 ml of adjusted methylene blue solution I to 100 ml with distilled water in a volumetric flask.

9.0 Sample Collection, Preservation and Handling

9.1 Samples must be taken with a minimum of aeration. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide to an unmeasurable form.

9.2 The analysis must be started immediately unless sample is preserved with 2N zinc acetate, 2 ml/1000 ml of sample and to a pH above 9 with 6N NaOH, 1 ml/1000ml of sample. Dissolved sulfides must be started immediately.

10.0 Calibration and Standardization

10.1 Concentrated sulfide solution preparation: Place several grams of clean, washed crystals of sodium sulfide ($Na_2S{\cdot}9H_2O$) in a small beaker. Add somewhat less than enough water to cover the crystals and stir occasionally for a few minutes. Pour the solution into another vessel. This reacts slowly with oxygen but the change is significant over a few hours. Make the solution daily.

10.2 Standardization of methylene blue I solution

10.2.1 To 1 liter of distilled water add 1 drop of the concentrated sulfide solution (10.1) and mix.

10.2.2 Immediately determine the sulfide concentration by the methylene blue procedure listed below and by the titrimetric iodine procedure (Method 376.1 in Methods for Chemical Analysis of Water and Wastes.)

10.2.3 Repeat using more than one drop of sulfide solution or less water until at least five tests have been made in the range of 1 to 8 mg/l sulfide.

10.2.4 Calculate the average percent error of the methylene blue procedure as compared to the titrimetric iodine procedure (method 376.1). Adjust by dilution or by adding more dye to methylene blue solution .

11.0 Procedure

11.1 Sample color development.

11.1.1 Transfer 7.5 ml of sample to each of two matched test tubes using a special wide tipped pipet or filling to a mark on the test tubes.

11.1.2 To tube A add 500 ul amine-sulfuric acid reagent and 150 ul $FeCl_3$ solution. Mix immediately by inverting the tube only once.

11.1.3 To tube B add 500 ul of 1+1 H_2SO_4 and 150 ul of the $FeCl_3$ solution and mix.

11.1.4 Color will develop in tube A in the presence of sulfide. Color development is usually complete in about 1 minute, but a longer time is often required for the fading of the initial pink color. Wait 3 to 5 minutes. Add 1.6 ml $(NH_4)_2HPO_4$ solution to each tube. Wait 3 to 5 minutes and make color comparisons. If zinc acetate was used for sample preservation wait at least 10 minutes before making comparisons.

11.2 Sample color comparison.

11.2.1 Visual: Add methylene blue solution I and/or II (depending on sulfide concentration and accuracy desired) dropwise to tube B until the color matches that developed in the first tube. If the concentration exceeds 20 mg/l repeat the dropwise addition of methylene blue solution using a portion of the sample diluted to one tenth.

11.2.2 Photometric: Use a 1 cm cell for 0.1 to 2.0 mg/l and a 10 cm cell for up to 20 mg/l. Zero the instrument with portion of the sample from tube B. Prepare a calibration curve from the data obtained in the methylene blue standardization, plotting concentration obtained from the titrimetric iodide procedure (method 376.1) versus absorbance. A straight line relationship can be assumed from 0 to 10 mg/L. Read the sulfide concentration from the calibration curve.

13.0 Data Analysis, Calculations, and Reporting Results

13.1 Visual comparison: With the methylene blue solution I adjusted so that 50ul = 1.0 mg/l sulfide and a 7.5 ml sample:

mg/l sulfide = microliters methylene blue solution I/50 +
(0.1) (microliters methylene blue solution II)/50.

13.2 Photometric: self explanatory, see section 11.2.2 above

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation such as ordering smaller quantities of standards or preparing reagents in smaller amounts that can be used completely. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories consult *Less is Better: Laboratory Chemical Management for Waste Reduction,* available form the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N. W. , Washington D.C. 20036, 202-872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15.2 For further information on waste management consult *The Waste Management Manual for Laboratory Personnel,* available from the American Chemical Society.

16.0 References

16.1 EPA-600/4-79-020, Methods for Chemical Analysis of Water and Wastes, revised March, 1983, pp. 376.2-1 through 376.2-3 (EPA Method 376.2).

16.2 Standard Methods For the Examination of Water and Wastewater, 20th Edition, 1998, Method 4500-S2-, pp. 4-165 to 4-166.